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March 09, 2005

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APPLICATION NUMBER: *60/608,522*
FILING DATE: *September 08, 2004*
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Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
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PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 319441440 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Quanlai	Song	Encinitas, CA

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MONOMERS FOR IMPROVED RNA AND DNA SOLUTION PHASE SYNTHESIS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 2 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☐ Other (specify) _____

☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

☑ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 500252

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Herb Boswell

TELEPHONE 760-603-2385

Date September 8, 2004

REGISTRATION NO. 27,311 *(if appropriate)*

Docket Number: DVCM0021US.L

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This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Additional Page

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Docket Number DVCM0021US.L

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Richard H.	Griffey	Vista, CA
Bruce S.	Ross	Carlsbad, CA

Number 2 of 2

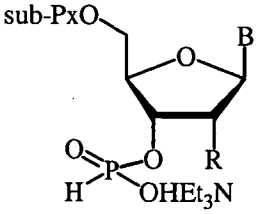
ROI-_____

4. DESCRIPTION OF INVENTION: (Pinpoint novel features: If new chemical compound - formula of generic concept, sample preparation from known starting materials. If new pharmaceutical composition or use - ingredients, dose regimens and preparation. If machine or chemical process - critical components and operation. Attach additional signed and dated pages or diagrams as necessary; indicate number of pages attached ___):_

As we have previously demonstrated, a convergent, solution phase synthesis of DNA via 3-6mer blocks of 5′-*O*-DMT protected H-phosphonate monomers is likely to be the most efficient and scalable method to produce commercial quantities of therapeutic oligonucleotides. This method can be further improved by combining our previously disclosed 5′ protecting group, the substituted pixyl. This new combination could be used in a further combination with various 2′-substitutions such as 2′-deoxy, 2′-O-alkyl (eg 2-methoxyethyl), 2′-deoxy-2′-halo (eg fluoro), 2′-protected hydroxyl (eg Cpep or tBDMS).

The use of substituted pixyl nucleoside derivatives over dimethoxytrityl (DMT) ones allow for certain advantages:

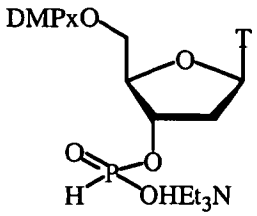
1. We have observed that pixyl derivatives are far easier to crystallize than the analogous DMT derivatives. Currently, each DMT derivative from monomer to each length of blockmer must be purified by silica gel chromatography. This limits the scale, increases the expense and potentially introduces newly generated degradation impurities. Purification by crystallization is usually superior, especially at production scale.
2. We can choose the substituted pixyl with the optimum acid stability for the base and sugar components in use. Both the pixyls and DMT are removed with acid. During this treatment, the rest of the oligonucleotide is vulnerable to degradation. The amount of degradation is dependent upon the acid strength and/or the exposure time. Notably deoxyadenosine and deoxyguanonsine will depurinate and acid sensitive RNA protecting groups such as Cpep will also degrade if there are any traces of water present. Furthermore, solution phase chemistry requires longer acid exposure time than solid phase synthesis. More acid sensitive pixyls will allow for less acid exposure and therefore allow less acid caused degradation.
3. Pixyl cations are known to be scavenged more efficiently than the DMT cation. As noted above, solution phase synthesis requires longer exposure to acidic deprotection conditions. The cation of the 5′-protecting group is in the same solution as the freed 5′-hydroxyl and is not washed away continuously as in the solid phase method. To minimize the reverse reaction, the cation can be scavenged and trapped with another nucleophile that can outcompete the 5′-hydroxyl. Reese has shown that adding pyrrole or triethylsilane efficiently traps pixyl cations and thus allows for even less exposure to acid.



Where B = A, C, G, U and their derivatives
and R = H, F, O-Me, O-CH2CH2OCH3, O-Cpep,O- Fpmp,
O-tBDMS, etc
and sub-Px = DMPx, etc

Example 1

Triethylammonium 5′-O-DMPx-thymidine 3′-H-phosphonate



Ammonium phenyl H-phosphonate (5.25g, 30 mmol), 5'-O-DMPx-thymidine (5.4 g, 10 mmol) and triethylamine (8.4 ml, 60 mmol) in pyridine (50 ml) were evaporated together under reduced pressure. The residue was coevaporated with dry pyridine (50 ml). The residue was dissolved in dry pyridine (50 ml) and the solution was cooled to 0oC. Pivaloyl chloride (3.7 ml, 30 mmol) was added dropwise over 10 min. After 30 min at 0oC, water (10 ml) was added and the stirred mixture was allowed to warm up to room temperature. Potassium phosphate buffer (1.0 M, pH 7.0, 250 ml) was added and the resulting mixture was concentrated under reduced pressureuntil all pyridine was removed. The residue was partitioned between dichloromethane (250 ml) and water (200ml). The organic layer was washed with triethylammonium phosphate buffer (0.5 m, pH 7, 3x100ml) and then evaporated. The residue was purified by a short silica gel column, eluted with dichloromethane-methanol (95:5 to 90:10). Evaporation of appropriate fractions to give the desired product (7.1 g).

5. UTILITY: (Describe the usefulness of the invention.)

The invention will allow us to produce DNA, DNA-substituted RNA hybrid, RNA, RNA-substituted RNA hybrid oligonucleotides for antisense, RNAi, miRNA applications on a commercial scale in a more efficient process and with higher purity.

6. WORKABLE EXTENT: (Describe plans for future work and envisaged variations of the invention.)

Future work first includes the synthesis of all our commonly used 2'-substituents (deoxy, methoxyethyl, methyl, fluoro, Cpep protected hydroxyl) as substituted pixyl H-phosphonate monomers. The monomers will be assembled into blocks and finally oligonucleotides of current interest in research and development.

7. REFERENCES: List particularly relevant references, including patents and publications
(To be done later)

Previous provisional application for substituted pixyls
Reese publication on blockmer synthesis, unpublished internal report on our blockmer syntheis
Reese publication on pixyls

8. COLLABORATION, SUPPORT:

Is there any corporate collaboration? ❑ Yes ✠ ✠ No If yes, name?_____

Is this research supported by a gov't grant (SBIR, etc.) ? ❑ Yes ✠ ✠ No Grant number